https://doi.org/10.31925/farmacia.2023.6.14 **ORIGINAL ARTICLE**

CHARACTERIZATION OF NOVEL CARPROFEN DERIVATIVES AS INHIBITORS OF THE SEROTONIN 5-HT2C RECEPTORS AND CLK4-KD PROTEIN KINASE FOR THE DEVELOPMENT OF PHARMACOLOGICALLY ACTIVE COMPOUNDS

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Abstract

In search of novel compounds with multiple biological activities, nine carprofen derivatives (**1a-i**) have been investigated by molecular docking to explore their interactions with target proteins involved in different diseases, from cancer to inflammation associated pathologies (viral infections, pain, neurodegenerative conditions). The docking studies have been performed to study the dual specificity for the protein kinase CLK4 and serotonin 2C (5-HT2C) receptor. Our molecular docking predictions indicate that the highest predicted binding energy has been observed in the case of the **1b** derivative for the 5-HT2C receptor and respectively in the case of derivative **1h** for CLK4-KD. Therefore, these two derivatives show a promising potential for the development of multi-pharmacologically active compounds.

Rezumat

În vederea descoperirii de noi compuși cu activități biologice multiple, nouă derivați ai carprofenului (**1a-i**) au fost investigați prin andocare moleculară pentru a explora interacțiunile lor cu proteinele țintă implicate în diferite boli, de la cancer la patologii asociate inflamației (infecții virale, durere, afecțiuni neurodegenerative). Studiile de andocare au fost efectuate pentru a studia specificitatea dual pentru receptorul proteinkinazei CLK4 și cel al serotoninei 2C (5-HT2C). Predicțiile noastre de andocare moleculară indică faptul că cea mai mare energie de legare prezisă a fost observată în cazul derivatului **1b** pentru receptorul 5-HT2C și, respectiv, în cazul derivatului **1h** pentru receptorul CLK4-KD. Prin urmare, acești doi derivați arată un potențial promițător pentru dezvoltarea compușilor cu potențial farmacologic multivalent.

Keywords: (3-4) carprofen derivatives, 5-HT2C receptors, CLK4-KD protein kinase

Introduction

Carbazole derivatives have multiple pharmacological activities such as anti-inflammatory [14, 32], antibacterial and antifungal [10, 25, 34], tuberculostatic [33, 48, 50], antimalarial [26, 36], antiviral [7], antioxidant [1, 29, 45], anticancer [24, 39, 56] and neuroprotective effects evidenced also in neurodegenerative conditions such as Alzheimer disease [15, 35, 41, 46]. Different research groups assessed the antiproliferative action of carbazoles on different types of tumoral cells. One of the mechanisms probably involved in this action is the ability of carbazoles to target the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, essential for cell differentiation, proliferation, development, apoptosis and inflammation, the STAT proteins representing therefore potential molecular targets for the discovery of new cancer drugs. Carbazoles could act by downregulating STAT proteins, and also by affecting interleukins and the inducible nitric oxide synthase production [12]. Zimmermann *et al*. designed and synthesized a series

of 9*H*-carbazole-1-carboxamides in order to evaluate their potential as selective ATP-competitive inhibitors of Janus kinase 2 (JAK2). 3-(1-Methyl-1*H*-indazol-5-yl)-7-(4-methylpiperazin-1-yl)-9*H*-carbazole-1-carboxamide has significant selectivity against JAK2, and also displayed an antiproliferative activity against SET-2 cells [59]. The studies of a small series of carbazoles N-alkylated with C5, C6 and C7 alkyl chains, namely dimethyl-5-(5-(6-methoxy-1,4-dimethyl-9*H*-carbazol-9-yl) pentyl/hexyl/heptyloxy) isophthalate, with potential STAT3 inhibitory activity, highlighted that the substituent of the carbazole nitrogen has a crucial role in modulating the lipophilic properties of the carbazole [47]. Also, Botta *et al*. synthesized a series of 1,4-dimethyl-carbazole derivatives and tested them for their potential activity on STAT3. Thus, there have been evaluated the possible effects on the cellular viability of human melanoma (A375) and human epithelial cervix adenocarcinoma (HeLa) cell lines, which constitutively express STAT3; 1,4-dimethyl-carbazole had similar activity to doxorubicin, used as a reference molecule, toward A375 cells [11]. 7-hydroxy-1-methyl-9*H*-carbazol-2-yl-5-(dimethylamino)-naphthalene-1-sulfonate inhibited the STAT3-mediated transcription and IL-6-induced phosphorylation of STAT3 in triple negative breast cancer (TNBC) cells and also suppressed cell proliferation in A431 (a squamous carcinoma cell line), A549 (a lung cancer cell line) and PC-3 (a prostate cancer cell line) [28]. Among substituted carbazole derivatives, synthesized by Baburajeev *et al.*, (3-acetyl-6-chloro-9*H*-carbazol-9-yl)methyl)-[1,10-biphenyl]-2-carbonitrile exhibited more important antiproliferative activity towards two lung cancer cell lines A549 and LLC [4]. From 35 studied compounds, bis(carbazole-2,9*N*-benzyl)-3-ethyl ethanoate (BC3EE2,9B) exhibited the most promising antiproliferative activity of two human glioblastoma multiforme cell lines, GBM8401 and GBM8901 cells, and induced the autophagy-mediated cell death. Anti-glioblastoma effects of synthetic carbazole derivative are promising because this compound sensitizes drug-resistant (temozolomide) glioblastoma cells [13]. The 6-(9-(2,4-Dichlorobenzyl)-9*H*-carbazol-3-yl)-N^2,N^2-dimethyl-3,6-dihydro-1,3,5-triazine-2,4-diamine was the less cytotoxic on gastric cancer cell lines (SGC-7901, AGS cells) and normal human liver cell line (L-02 cells). (*E*)-2-((9-(4-Methylbenzyl)-9*H*-carbazol-3-yl)methylene)hydrazine-1-carboximidamide also exhibited anticancer therapeutic potential. Therefore, the antitumour potential of these carbazole derivatives could be further explored for future therapeutic applications [57]. By combining the carbazole moiety with heterocycles such as piperazine and imidazole, new topoisomerase-I inhibitors have been developed. The presence of an electron-releasing group on the benzene ring, as well as the hydrogen attached to the carbazole nitrogen atom which is involved in the formation of the hydrogen bond with the active site of topoisomerase I, increase the potency of the new derivatives. The substitution at the nitrogen atom in position 9 of carbazole moiety also increases the therapeutic value of carbazole derivatives toward the treatment of cancer [31]. Also, new N-substituted carbazole derivatives have been screened for their neuroprotective activities. The results of the antioxidant activity screening have revealed that the compound 2-phenyl-9-(*p*-tolyl)-9*H*-carbazole was the most potent and has significantly prevented cell death through a glutathione-independent way. Due to its low toxicity and neuroprotective effects, the compound 2-phenyl-9-(*p*-tolyl)-9*H*-carbazole could be a potential lead compound for further development of novel agents with potent neuroprotection against oxidative stress-associated central nervous system diseases such as Alzheimer disease [58]. Promising candidates for the treatment of neurodegenerative diseases can be conjugates of aminoadamantanes with carbazole derivatives. These compounds act by inhibiting butyrylcholinesterase, blocking NMDA receptors, exhibiting microtubules stabilizing properties and protecting nerve cells from death at the calcium overload conditions [5].

Different derivatives with a carbazole nucleus obtained through a series of structural modifications of natural compounds were analysed in order to highlight the possibility of an antiviral action. An example is represented by N-alkyl-pyrido[4,3-c]carbazole derivatives that were synthesized by Hirata *et al*., starting from a constituent (mukonal) that can be found in plants from the *Rutaceae* family. They are structurally related to the alkaloid ellipticine, as well as to its synthetic analogues that exhibit antiviral activity. Among these derivatives, some showed inhibitory action on HIV replication and 5-methoxy-7-methyl-7H-pyrido[3,4-c]carbazole has the highest therapeutic index [23].

The carprofen scaffold is a source for new effective and safe analgesic and anti-inflammatory drugs. Carprofen, 2-(6-chloro-9*H*-carbazol-2-yl)propanoic acid, was identified as a multi-target fatty acid amide hydrolase (FAAH)/cyclooxygenase (COX) hit. The concomitant inhibition of these enzymes improved the analgesic response and had low side effects in animal models of pain. This is the reason why Favia *et al*. designed and synthesized racemic derivatives of carprofen by functionalisation of the acid moiety, by insertion of the nitrogen functional groups and by removal of the chlorine atom of the carprofen. These compounds showed a significant multi-target inhibitory activity and may be a starting point for the discovery of new FAAH/COXs inhibitors used in the treatment of pain and inflammation [22]. Following a study by Bandgar *et al*., 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1*H*-2-pyrazoline derivatives were synthesized and analysed *in vitro* to highlight their anti-inflammatory and antioxidant actions. The ability to inhibit the two cyclooxygenase isoforms was evaluated for the anti-inflammatory action. Thus, from the 15 obtained

compounds, the derivatives with the phenyl radical substituted with methoxy group(s) and the derivative with the thiophenic nucleus, strongly inhibited cyclo-oxygenase 2 (COX 2). A considerable increase of the inhibitory action on COX 2 was obtained by replacing the aryl with a heterocyclic radical. The selectivity towards COX 2 was anticipated, since the synthesized compounds have a carprofen-like structure. Regarding the antioxidant action, the ability to inhibit superoxide and hydroxyl radicals was tested. Almost all synthesized compounds inhibited the superoxide radical, their capacity being compared to that of ascorbic acid, which is considered a standard. It was highlighted that the derivatives whose aryl radical is substituted with two halogen atoms are more potent compared to the mono-halogenated ones. Also, regarding the inhibition of the hydroxyl radical, the disubstituted derivatives were more potent [6]. Starting from the idea that compounds which are able to inhibit FAAH and COX may be potentially useful therapeutic agents and which have the advantage that inhibition of FAAH reduces the gastrointestinal damage produced by COX-inhibiting non-steroidal anti-inflammatory drugs, novel amide analogues of carprofen as potential FAAH/ COX dual action inhibitors were investigated, and it was concluded that 2-(6-chloro-9*H*-carbazol-2-yl)-N-(3-methylpyridin-2-yl)propenamide and 2-(6-chloro-9*H*-carbazol-2-yl)-N-(3-chloropyridin-2-yl)propenamide are dual-acting FAAH/substrate-selective COX inhibitors [17].

The pathophysiology of various human mental diseases, including schizophrenia, anxiety and major depression, is frequently connected to the serotonin receptor (5-HT) family [30]. The 5-HT2C is a G protein-coupled receptor that binds the neurotransmitter serotonin. The activation of this receptor by serotonin reduces dopamine and regulates various behaviours, including anxiety and mood, consequently, this receptor may represent a therapeutic target [49]. The CLKs (Cyclin dependent-like family kinases) are dual-specificity protein kinases involved in the transcript splicing by mechanism of phosphorylation of serine and arginine-rich proteins. Their activity consists in catalysing spliceosome molecular machinery and modulating the activities or expression of non-splicing proteins, biochemical processes which are correlated with different diseases. One of these CLKs isoforms CLK4-KD is involved in the phosphorylation of serine, arginine, threonine and tyrosine residues and is crucial in the control of fundamental cellular activities. This kinase may be a target for several diseases, including cancer, Duchenne muscular dystrophy, inflammatory and viral diseases, or neurodegenerative diseases [2, 16, 38, 40, 51]. Despite these considerations, just a few numbers of computer-aided molecular design and discovery studies focused on CLK4 and 5-HT2C receptors modulators were reported.

In this study, using the molecular docking approach, we predict the interaction between 5-hydroxytryptamine receptor 2C (5-HT2C) as well the dual specificity for the protein kinase CLK4 (CLK4-KD) of previously synthesized and characterized new carprofen derivatives. The main reasons for the synthesis of new carbazole derivatives were their multiple biological properties as well as the recent observation of the phenomenon of iso-structurality for two pairs of halogenated carprofens [20] and our ongoing studies of halogen bonding of halogenated aromatic N-heterocyclic compounds [8, 9, 18, 21, 43, 44].

Materials and Methods

Molecular Docking approach

We have prepared the derivatives and the proteins for the molecular docking approach in accordance with our standard molecular docking protocol [3, 55]. We have imported the experimentally-determined 3D structures of 5-HT2C and CLK4-KD proteins from the Protein Data Bank (PDB) [53]. The structure of 5-HT2C (PDB: 6BQH) is in complex with ritanserin in the binding site, while CLK4-KD (PDB: 6FYV) is in complex with 5-[(3-chlorophenyl)amino]benzo[c]-[2,6]naphthyridine-8-carboxylic acid (3NG) compound in the binding site [27, 42].

The grid parameters were created to only include the proteins binding sites; they are shown in Table I.

Table I

The parameters of the Grid Box for both 5-HT2C (PDB: 6BQH) CLK4-KD (PDB: 6FYV)

Protein	Grid Point Spacing	Specified Grid Points	Coordinates of Central Grid Point of Maps
5-HT2C	0.375	X-points 58, Y- points 58 Z-points 58	40.849, 29.447, 57.746
CLK4-KD	0.375	X-points 60, Y- points 60 Z-points 60	-25.556, 25.401, -20.070

For the molecular docking approach, we have used Autodock 4.2.6. software [37]. The hybrid Lamarckian-Genetic search algorithm was used to predict the binding affinity of the derivatives **1a**–**1i**. For each protein-ligand conformation, we have made 100 runs [54].

Computational Pharmacodynamic Profiles of the compounds 1a-1i and molecular docking

From the SuperPred database, we extracted the most significant molecular targets for compounds **1a- 1i**. We selected the most relevant targets CLK4 and 5-

HT2C, with probability (%) of interactions and model accuracy (%). We noticed that regarding (i) CLK4 the probability of interactions was from 100% (**1a** and **1i**) and 89.71% (**1f**) and (ii) 5-HT2c, receptor CLK4 the probability of interactions was from 96% (**1f**) and 77% (**1h**) (Table II).

Table II

The molecular targets of their UNIPROT and PDB code, probability of interactions and model accuracy of complexes

Target name	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**
Dual specificity protein kinase CLK4 (UNIPROT- Q9HAZ1, ped(6FYV)									
Probability (%)	100	96.58	97.85	97.64	93.3	89.71	93	99	100
Model accuracy (%)	94	94.45	94.45	94.45	94	94.75	94	94	94
Serotonin 2c (5-HT2c) receptor (UNIPROT- Q9HAZ1, ped(6FYV)									
Probability (%)	81%	88.16%	90.94%	92.62%	95%	96%	85%	77%	87%
Model accuracy (%)	90%	89.62%	89.62%	89.62%	90%	90%	90%	90%	90%

Results and Discussion

Chemistry

In a previously published study [19], we presented the synthesis of same compounds that have pharmacologically active heterocyclic carbazoles, the proof of their structure with FT-IR and NMR spectral analyses, the elemental analysis, as well as drug-likeness, pharmacokinetics and pharmacogenomics profiles of compounds, total antioxidant capacity measurements and antimicrobial activity.

The chemical structure of new carbazole derivatives synthesized from the NSAID carprofen are presented in Figure 1.

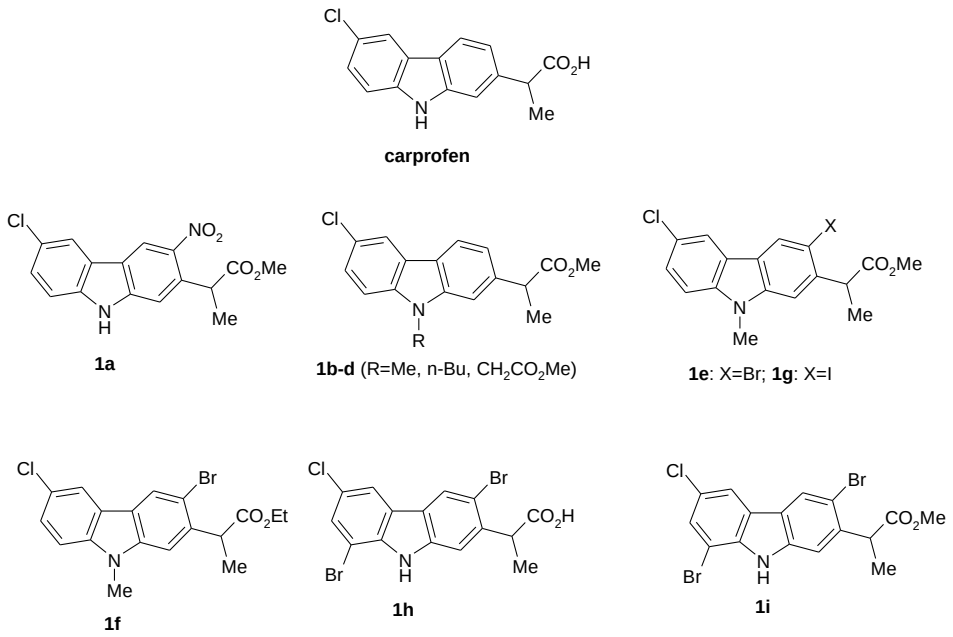

Figure 1.

The chemical structure of new carbazole derivatives synthesized from the NSAID carprofen

Molecular Docking study

The interaction of the compounds with the amino-acid (aa) residues was analysed. According to the PDB structure, ritanserin interacts with TRP130, ASP134, VAL135, SER138, VAL208, VAL215, GLY218, ALA222, TRP324, LEU350, ASN351, VAL354, TYR358, aa residues and HOH1301 from 5-HT2C protein [42]. According to our predictions, derivatives **1a**-**1c** bind similarly to the drug ritanserin to the 5-HT2C receptor. As seen in Figure 2, these derivatives show interactions with aa residues similar to ritanserin at the binding site (Figure 2).

When interacting with 5-HT2C receptor, derivative **1d** showed the lowest predicted binding energy (-11.44 kcal/mol). This compound has similar interactions with 5-HT2C receptor just like ritanserin (Figure 2). Both compounds form alkyl interactions with VAL135 and ALA222 aa residues, Pi-Pi Stacked interactions with TRP324 aa. Also, both compounds interact with VAL215 and GLY218 aa residues (Figure 2).

The 3NG ligand was co-crystallized with the CLK4-KD [25]. This compound forms interactions with LEU167, GLY168, GLU169, PHE172, VAL175, ALA189, LYS191, VAL225, PHE 241, LEU244, LEU295, VAL324, ASP325 aa residues and forms two Water H-Bonds with HOH601 and HOH628 (Figure 3).

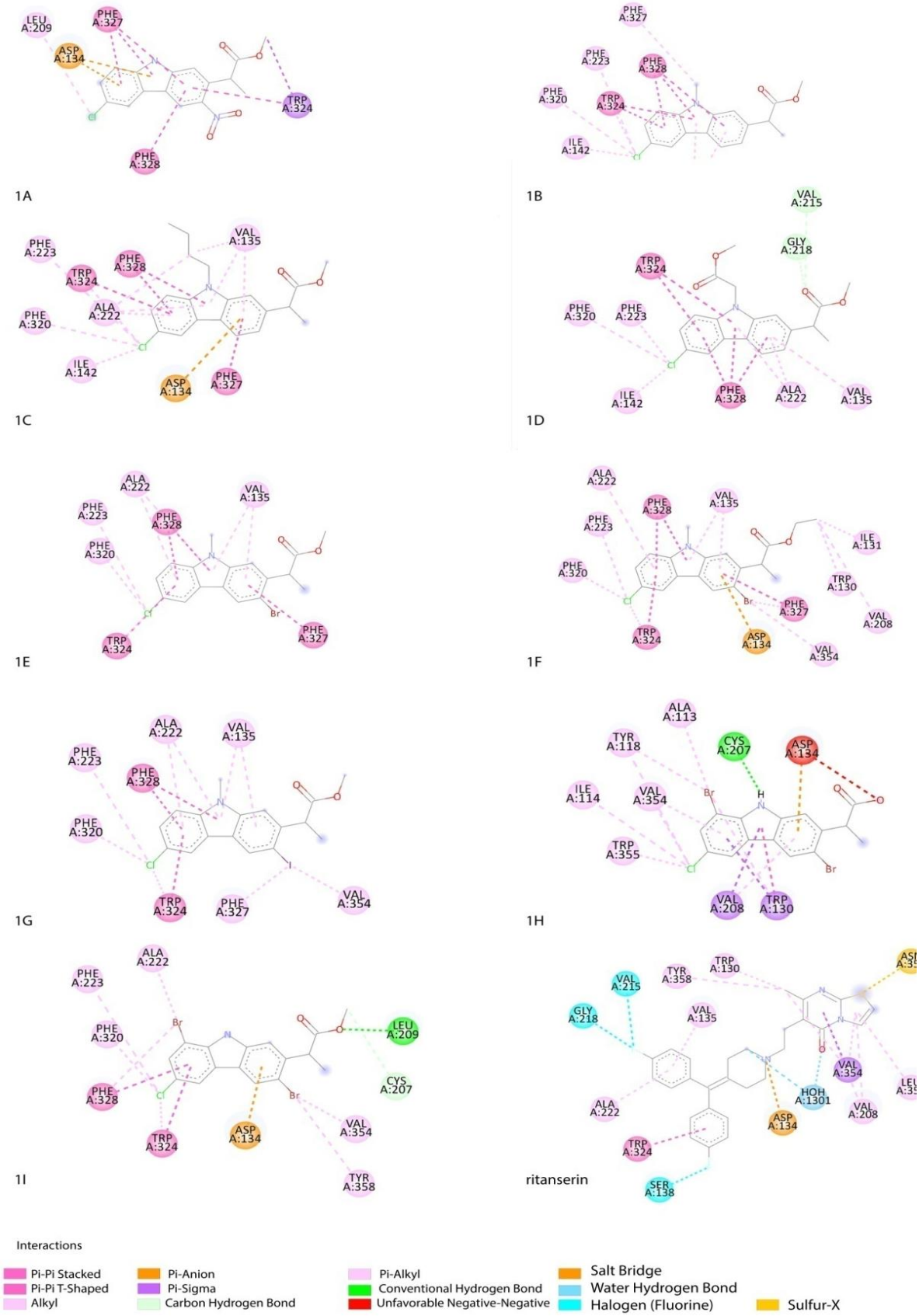

Figure 2.

The predicted interactions between **1a-1i** derivatives and 5-HT2C receptor, compared with ritanserin determined interactions

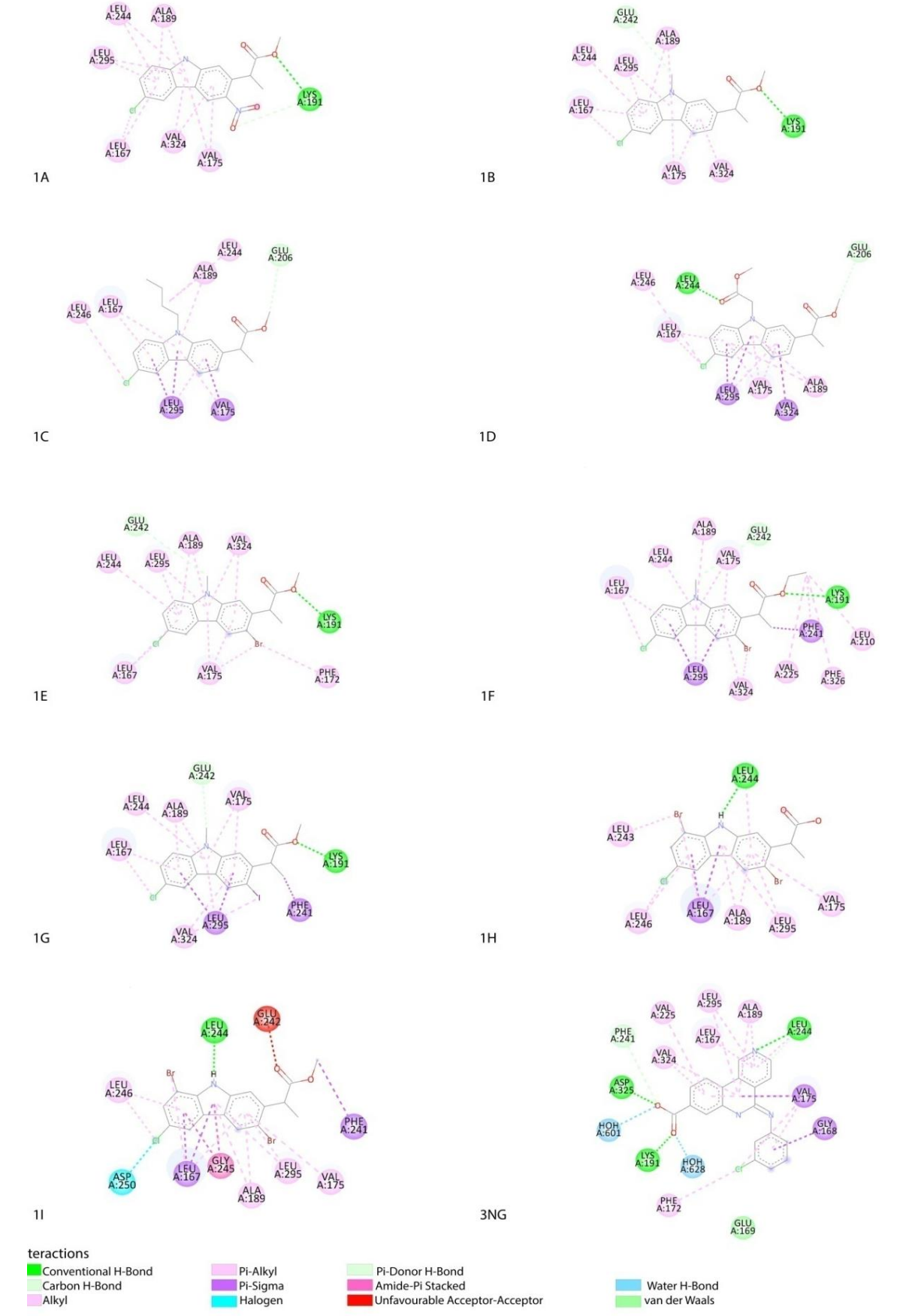

Figure 3.
The predicted interactions between **1a-1i** derivatives and CLK4-KD compared with 3NG determined interactions

The lowest predicted binding energy is between derivative **1g** and CLK4-KD (-10.84 kcal/mol). This derivative interacts with LEU167, ALA189 and VAL324 *via* alkyl and pi-alkyl interactions; additionally, both compounds interact *via* an H-Bond interaction with LYS191 amino acid.

The similar interactions with the amino acid residues between the **1a**-**1i** derivatives and the co-crystalized ligand structures 3NG and ritanserin indicate that those derivatives may bind similar to CLK4-KD and 5-HT2C proteins (Figure 2 and Figure 3).

A predicted binding energy lower than -6 kcal/mol indicates a favourable interaction between the compound and target [52]. Our results indicate that the **1a**-**1i** derivatives present favourable binding energies when interacting with both 5-HT2C and CLK4-KD (Table III).

Table III

The predicted binding energy between **1a**-**1i** derivatives and proteins 5-HT2C respectively

Compound Name	5-HT2C (PDB code: 6BQH) lowest binding energy (kcal/mol)	CLK4-KD (PDB code: 6FYV) lowest binding energy (kcal/mol)
1a	-10.75	-10.72
1b	-9.99	-10.82
1c	-10.59	-10.19
1d	-11.44	-11.46
1e	-10.29	-10.68
1f	-10.81	-10.67
1g	-10.41	-10.84
1h	-10.05	-10.00
1i	-10.10	-10.60

Conclusions

In search of novel compounds with biological activities, nine carprofen derivatives have been investigated by molecular docking to explore the interactions of these carprofen derivatives with target proteins involved in different diseases, from cancer to inflammation associated pathologies (viral infections, pain, neuro-degenerative conditions).

Our molecular docking predictions indicate that the highest predicted binding energy has been observed in the case of the **1b** derivative for the 5-HT2C receptor and respectively in the case of derivative **1h** for CLK4-KD. Therefore, these two derivatives show a promising potential for the development of multi-pharmacologically active compounds.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Al-Majidi MHMA-D, Al-Majidi SMH, The synthesis, identification, and evaluation of some new antioxidant activities β-lactam from N-carbazole derivatives. *Eurasian Chem Commun.*, 2022; 4(3): 209-221.
2. Al-Tawil MF, Daoud S, Ma'mon MH, Taha MO, Discovery of new Cdc2-like kinase 4 (CLK4) inhibitors *via* pharmacophore exploration combined with flexible docking-based ligand/receptor contact fingerprints and machine learning. *RSC Adv*., 2022; 12(17): 10686-10700.
3. Avram S, Duda-Seiman D, Borcan F, Wolschann P, QSAR-CoMSIA applied to antipsychotic drugs with their dopamine D2 and serotonine 5HT2A membrane receptors. *J Serbian Chem Soc.*, 2011; 76(2): 263-281.
4. Baburajeev CP, Mohan CD, Patil GS, Rangappa S, Pandey V, Sebastian A, Fuchs JE, Bender A, Lobie PE, Basappa S, Rangappa KS, Nano-cuprous oxide catalyzed one-pot synthesis of a carbazole-based STAT3 inhibitor: A facile approach *via* intramolecular C-N bond formation reactions. *RSC Adv*., 2016; 6(43): 36775-36785.
5. Bachurin SO, Shevtsova EF, Makhaeva GF, Grigoriev VV, Boltneva NP, Kovaleva NV, Lushchekina SV, Shevtsov PN, Neganova ME, Redkozubova OM, Bovina EV, Gabrelyan AV, Fisenko VP, Sokolov VB, Aksinenko AY, Echeverria V, Barreto GE, Aliev G, Novel conjugates of aminoadamantanes with carbazole derivatives as potential multitarget agents for AD treatment. *Sci Rep.*, 2017; 7(1): 45627.
6. Zimmar BP, Adsul LK, Chavan HV, Jalde SS, Shringare SN, Shaikh R, Meshram RJ, Gacche RN, Masand V, Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1H-2-pyrazolines as potent anti-inflammatory and antioxidant agents. *Bioorg Med Chem Lett.*, 2012; 22(18): 5839-5844.
7. Bonomo MG, Caruso A, El-Kashef H, Salzano G, Sinicropi MS, Saturnino C, An Update of Carbazole Treatment Strategies for COVID-19 Infection. *Appl Sci.*, 2023; 13: 1522.
8. Bordei (Telehoiu) AT, Nuță DC, Mușat GC, Missir AV, Căproiu MT, Dumitrașcu F, Zarafu I, Ioniță P, Bădiceanu CD, Limban C, Ozon EA, Microwave assisted synthesis and spectroscopic characterization of some novel Schiff bases of carprofen hydrazide. *Farmacia*, 2019; 67(6): 955-962.
9. Bordei (Telehoiu) AT, Costea T, Limban C , Nuță DC, Gîrd CE, Zarafu I, Balaci TD , Karampelas O, Ancuceanu RV, New insights on some 6-chloro-9h-

carbazol derivatives concerning their *in vitro* antioxidant capacity and *in vivo* cytotoxicity. *Farmacia*, 2021; 69(3): 475-480.
10. Bordei (Telehoiu) AT, Limban C, Nuță DC, Zarafu I, Denes E, Măruțescu L, Chifiriuc MC, Popa M, Aramă A, Recent advances in the study of derivatives of (EZ)-N'- benzylidene-(2RS)-2-(6-chloro-9H-carbazol-2-yl) propano-hydrazide, *Farmacia*, 2022, 70(4), 589-595.
11. Botta A, Sirignano E, Popolo A, Saturnino C, Terracciano S, Foglia A, Sinicropi MS, Longo P, Di Micco S, Identification of lead compounds as inhibitors of STAT3: design, synthesis and bioactivity. *Mol Inform.*, 2015; 34(10): 689-697.
12. Caruso A, Barbarossa A, Carocci A, Salzano G, Sinicropi MS, Saturnino C, Carbazole derivatives as STAT inhibitors: An overview. *Appl Sci.*, 2021; 11(13): 6192.
13. Chen CM, Syu JP, Way TD, Huang LJ, Kuo SC, Lin CT, Lin CL, BC3EE2, 9B, a synthetic carbazole derivative, upregulates autophagy and synergistically sensitizes human GBM8901 glioblastoma cells to temozolomide. *Int J Mol Med.*, 2015 ;36(5): 1244-1252.
14. Chen Y, Cao N, Lv H, Zeng K, Yuan J, Guo X, Zhao M, Tu P, Jiang Y, Anti-inflammatory and cytotoxic carbazole alkaloids from *Murraya kwangsiensis*. *Phytochemistry*, 2020; 170: 112186.
15. Choubdar N, Golshani M, Jalili-Baleh L, Nadri H, Küçükkilinç TT, Ayazgök B, Moradi A, Moghadam FH, Abdolahi Z, Ameri A, Salehian F, Foroumadi A, Khoobi M, New classes of carbazoles as potential multi-functional anti-Alzheimer's agents. *Bioorg Chem.*, 2019; 91: 103164.
16. Coombs TC, Tanega C, Shen M, Wang JL, Auld DS, Gerritz SW, Schoenen FJ, Thomas CJ, Aubé J, Small-molecule pyrimidine inhibitors of the cdc2-like (Clk) and dual specificity tyrosine phosphorylation-regulated (Dyrk) kinases: development of chemical probe ML315. *Bioorg Med Chem Lett.*, 2013; 23(12): 3654-3661.
17. Deplano A, Karlsson J, Fowler CJ, Onnis V, The fatty acid amide hydrolase and cyclooxygenase-inhibitory properties of novel amide derivatives of carprofen. *Bioorg Chem.*, 2020; 101: 104034.
18. Draghici C, Caira MR, Dumitrescu DE, Dumitrascu F, Halogen Bonds of 4-Iodosydnones in Solution Deduced from 13C-NMR Spectra. *Rev Chem.*, 2018; 69: 843-845.
19. Dumitrascu F, Caira MR, Avram S, Buiu C, Udrea AM, Vlad IM, Zarafu I, Ioniță P, Nuță DC, Popa M, Chifiriuc MC, Limban C, Repurposing anti-inflammatory drugs for fighting planktonic and biofilm growth. New carbazole derivatives based on the NSAID carprofen: synthesis, *in silico* and *in vitro* bioevaluation. *Front Cell Infect Microbiol.*, 2023; 13: 1181516.
20. Dumitrascu F, Udrea AM, Caira MR, Nuta DC, Limban C, Chifiriuc MC, Popa M, Bleotu C, Hanganu A, Dumitrescu D, Avram S, *In Silico* and Experimental Investigation of the Biological Potential of Some Recently Developed Carprofen Derivatives. *Molecules*. 2022; 27(9): 2722.
21. Dumitrescu D, Shova S, Man IC, Caira MR, Popa MM, Dumitrascu F, 5-Iodo-1-Arylpyrazoles as Potential Benchmarks for Investigating the Tuning of the Halogen Bonding. *Cryst.*, 2020; 10(12): 1149.
22. Favia AD, Habrant D, Scarpelli R, Migliore M, Albani C, Bertozzi SM, Dionisi M, Tarozzo G, Piomelli D, Cavalli A, De Vivo M, Identification and Characterization of Carprofen as a Multitarget Fatty Acid Amide Hydrolase/Cyclooxygenase Inhibitor. *J Med Chem.*, 2012; 55(20): 8807-8826.
23. Hirata K, Ito C, Furukawa H, Itoigawa M, Cosentino LM, Lee KH, Substituted 7H-pyrido [4,3-c] carbazoles with potent anti-HIV activity. *Bioorg Med Chem Lett.*, 1999; 9(2): 119-122.
24. Issa S, Prandina A, Bedel N, Rongved P, Yous S, Le Borgne M, Bouaziz Z, Carbazole scaffolds in cancer therapy: a review from 2012 to 2018. *J Enzyme Inhib Med Chem.*, 2019; 34(1): 1321-1346.
25. Kadnor VA, Shelke SN, Synthesis, antimicrobial and antimalarial activity of 1,4-benzothiazepine and pyrazolinederivatives incorporating carbazolemoiety. *Bulg Chem Commun.*, 2019; 51(2): 234-241.
26. Kadnor VA, Shirole GD, Mhaske GR, Shelke SN, Synthesis, antimicrobial and antimalarial activities of some carbazole derivatives. *Indian J Heterocycl Chem.*, 2017; 27(1): 59-64.
27. Kallen J, Bergsdorf C, Arnaud B, Bernhard M, Brichet M, Cobos-Correa A, Elhajouji A, Freuler F, Galimberti I, Guibourdenche C, Haenni S, Holzinger S, Hunziker J, Izaac A, Kaufmann M, Leder L, Martus HJ, von Matt P, Polyakov V, Roethlisberger P, Roma G, Stiefl N, Uteng M, X-ray Structures and Feasibility Assessment of CLK2 Inhibitors for Phelan-McDermid Syndrome. *ChemMedChem.*, 2018; 13(18): 1997-2007.
28. Kang HJ, Yi YW, Hou S-J, Kim HJ, Kong Y, Bae I, Brown ML, Disruption of STAT3-DNMT1 interaction by SH-I-14 induces re-expression of tumor suppressor genes and inhibits growth of triple-negative breast tumor. *Oncotarget*, 2017; 8(48): 83457.
29. Karaaslan C, Ince E, Gurer-Orhan H, Tavakkoli M, Firuzi O, Saso L, Suzen S, Behaviour of 9-Ethyl-9H-carbazole hydrazone derivatives against oxidant systems: Protective effect on amyloid β-induced damage. *Croat Chem Acta*, 2019; 92(1): 87-94.
30. Kitson SL, 5-hydroxytryptamine (5-HT) receptor ligands. *Curr Pharm Des.*, 2007; 13(25): 2621-2637.
31. Kumar N, Pathak D, Design, synthesis and anticancer activity of 9-substituted carbazole derivatives. *Int J Pharm Sci Res.*, 2016; 7(8): 3291.
32. Kumar U, Kumar Gupta S, Yogi B, Kumar Gautam S, Synthesis and evaluation of biological activity of some novel carbazole derivatives. *Indian J Chem.*, 2023; 62: 60-64.
33. Ma C, Case RJ, Wang Y, Zhang HJ, Tan GT, Hung NV, Cuong NM, Fransblau SG, Soejarto DD, Fong HH, Pauli GF, Anti-tuberculosis constituents from the stem bark of Micromelum hirsutum. *Planta Med.*, 2005; 71(3): 261-267.
34. Merzouki O, Arrousse N, El Barnossi A, Ech-chihbi E, Fernine Y, Housseini IA, Rais Z, Taleb M, Eco-friendly synthesis, characterization, *in-silico* ADMET and molecular docking analysis of novel carbazole derivatives as antibacterial and antifungal agents. *J Mol Struct.*, 2023; 1271: 133966.

35. Miura H, Takano K, Kitao Y, Hibino S, Choshi T, Murakami R, Suzuki H, Yamada M, Ogawa S, Hori O, A carbazole derivative protects cells against endoplasmic reticulum (ER) stress and glutathione depletion. *J Pharmacol Sci.*, 2008; 108(2): 164-171.
36. Molette J, Routier J, Abla N, Besson D, Bombrun A, Brun R, Burt H, Georgi K, Kaiser M, Nwaka S, Muzerelle M, Scheer A, Identification and optimization of an aminoalcohol-carbazole series with antimalarial properties. *ACS Med Chem Lett.*, 2013; 4(11): 1037-1041.
37. Morris GM, Huey R, Olson AJ, Using AutoDock for Ligand-Receptor Docking. *Curr Protoc Bioinformatics*, 2008; 24(1): Chapter 8: Unit 8.14.
38. Myers MP, Murphy MB, Landreth G, The dual-specificity CLK kinase induces neuronal differentiation of PC12 cells. *Mol Cell Biol.*, 1994; 14(10): 6954-6961.
39. Nguyen MAT, Mungara AK, Kim JA, Lee KD, Park S, Synthesis, anticancer and antioxidant Activity of novel carbazole-based thiazole derivatives. *Phosphorus Sulfur Silicon Relat Elem.*, 2015; 190(2): 191-199.
40. Pan Y, Wang Y, Bryant SH, Pharmacophore and 3D-QSAR characterization of 6-arylquinazolin-4-amines as Cdc2-like kinase 4 (Clk4) and dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) inhibitors. *J Chem Inf Model.*, 2013; 53(4): 938-947.
41. Patel KB, Patel DV, Patel NR, Kanhed AM, Teli DM, Gandhi B, Shah BS, Chaudhary BN, Prajapati NK, Patel KV, Yadav MR, Carbazole-based semicarbazones and hydrazones as multifunctional anti-Alzheimer agents. *J Biomol Struct Dyn.*, 2022; 40(20): 10278-10299.
42. Peng Y, McCorvy JD, Harpsøe K, Lansu K, Yuan S, Popov P, Qu L, Pu M, Che T, Nikolajsen LF, Huang XP, Wu Y, Shen L, Bjørn-Yoshimoto WE, Ding K, Wacker D, Han GW, Cheng J, Katritch V, Jensen AA, Hanson MA, Zhao S, Gloriam DE, Roth BL, Stevens RC, Liu ZJ, 5-HT2C Receptor Structures Reveal the Structural Basis of GPCR Polypharmacology. *Cell*, 2018; 172(4): 719-730.e14.
43. Popa MM, Man IC, Draghici C, Shova S, Caira MR, Dumitrascu F, Dumitrescu D, Halogen bonding in 5-iodo-1-arylpyrazoles investigated in the solid state and predicted by solution 13 C-NMR spectroscopy. *CrystEngComm.*, 2019; 21(46): 7085-7093.
44. Popa MM, Shova S, Hrubaru M, Barbu L, Draghici C, Dumitrascu F, Dumitrescu D, Introducing chirality in halogenated 3-arylsydnones and their corresponding 1-arylpyrazoles obtained by 1, 3-dipolar cycloaddition. *RSC Adv.*, 2020; 10(27): 15656-15664.
45. Reddy SVL, Naresh K, Raju CN, New sulfonamide and carbamate derivatives of 4-(oxiran-2-ylmethoxy)-9*H*-carbazole: Synthesis, characterization, antimicrobial and antioxidant activities. *Der Pharm Lett.*, 2013; 5: 221-231.
46. Saturnino C, Iacopetta D, Sinicropi MS, Rosano C, Caruso A, Caporale A, Marra N, Marengo B, Pronzato MA, Parisi OI, Longo P, Ricciarelli R, N-alkyl carbazole derivatives as new tools for Alzheimer's disease: Preliminary studies. *Molecules*, 2014; 19(7): 9307-9317.
47. Saturnino C, Palladino C, Napoli M, Sinicropi MS, Botta A, Sala M, Carcereri de Prati A, Novellino E, Suzuki H, Synthesis and biological evaluation of new N-alkylcarbazole derivatives as STAT3 inhibitors: preliminary study. *Eur J Med Chem.*, 2013; 60: 112-119.
48. Sellamuthu S, Gutti G, Kumar D, Kumar Singh S, Carbazole: A Potent Scaffold for Antitubercular Drugs. *Mini Rev Org Chem.*, 2018; 15(6): 498-507.
49. Serretti A, Artioli P, De Ronchi D, The 5-HT2C receptor as a target for mood disorders. *Expert Opin Ther Targets.*, 2004; 8(1): 15-23.
50. Shaikh MS, Kanhed AM, Chandrasekaran B, Palkar MB, Agrawal N, Lherbet C, Hampannavar GA, Karpoormath R, Discovery of novel N-methyl carbazole tethered rhodanine derivatives as direct inhibitors of *Mycobacterium tuberculosis* InhA. *Bioorganic Med Chem Lett.*, 2019; 29(16): 2338-2344.
51. Song M, Pang L, Zhang M, Qu Y, Laster KV, Dong Z, Cdc2-like kinases: structure, biological function, and therapeutic targets for diseases. *Signal Transduct Target Ther.*, 2023; 8(1): 148.
52. Tozar T, Santos Costa S, Udrea AM, Nastasa V, Couto I, Viveiro M, Pascu ML, Romanitan MO, Anti-staphylococcal activity and mode of action of thioridazine photoproducts. *Sci Rep.*, 2020; 10(1): 1-12.
53. Udrea AM, Mernea M, Buiu C, Avram S, *Scutellaria baicalensis* Flavones as Potent Drugs against Acute Respiratory Injury during SARS-CoV-2 Infection: Structural Biology Approaches. *Processes*, 2020; 8(11): 1468.
54. Udrea AM, Avram S, Nistorescu S, Pascu ML, Romanitan MO, Laser irradiated phenothiazines: New potential treatment for COVID-19 explored by molecular docking. *J Photochem Photobiol B Biol.*, 2020; 211: 111997.
55. Udrea AM, Dinache A, Pagès JM, Pirvulescu RA, Quinazoline derivatives designed as efflux pump inhibitors: Molecular modeling and spectroscopic studies. *Molecules*, 2021; 26(8): 2374.
56. Wang G, Sun S, Guo H, Current status of carbazole hybrids as anticancer agents. *Eur J Med Chem.*, 2022; 229: 113999.
57. Xue YJ, Li MY, Jin XJ, Zheng CJ, Piao HR, Design, synthesis and evaluation of carbazole derivatives as potential antimicrobial agents. *J Enzyme Inhib Med Chem.*, 2021; 36(1): 295-306.
58. Zhu D, Chen M, Li M, Luo B, Zhao Y, Huang P, Xue F, Rapposelli S, Pi R, Wen S, Discovery of novel N-substituted carbazoles as neuroprotective agents with potent anti-oxidative activity. *Eur J Med Chem.*, 2013;68: 81-88.
59. Zimmermann K, Sang X, Mastalerz HA, Johnson WL, Zhang G, Liu Q, Batt D, Lombardo LJ, Vyas D, Trainor GL, Tokarski JS, Lorenzi MV, You D, Gottardis MM, Lippy J, Khan J, Sack JS, Purandare AV, 9*H*-Carbazole-1-carboxamides as potent and selective JAK2 inhibitors. *Bioorg Med Chem Lett.*, 2015; 25(14): 2809-2812.